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EFFECT OF PULSED 5.62 GHz MICROWAVES ON SQUIRREL MONKEYS (*Saimiri sciureus*) PERFORMING A REPEATED ACQUISITION TASK

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NAVAL AEROSPACE MEDICAL RESEARCH LABORATORY

PENSACOLA FLORIDA

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EFFECT OF PULSED 5.62 GHz MICROWAVES ON SQUIRREL MONKEYS (*Saimiri sciureus*) PERFORMING A REPEATED ACQUISITION TASK

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Naval Medical Research and Development Command
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SUMMARY*

THE PROBLEM

Navy personnel assigned to perform duties in the vicinity of microwave irradiating devices are subject to possible hazards if the irradiation is of adequate intensity and frequency. Data are of critical need to establish safety standards for human exposure to microwaves. Such information cannot be gained from human studies because of the potential hazards, but fairly reliable and valid results from non-human primate experiments may be extrapolated to man and then used for setting human safety standards. In an effort to provide such information squirrel monkeys were trained on a task that required repeated learning and were subsequently irradiated with microwaves while they performed the task.

FINDINGS

Four male squirrel monkeys trained to perform a repeated learning task demonstrated performance decay while being exposed to pulsed 5.62 GHz microwave radiation in the far-field situation at power densities of 38 and 46 mW/cm^2, but there was only slight learning impairment. There was little, if any, effect on learning or performance at 17 and 32 mW/cm^2. The performance effect became evident when the monkey's colonic temperature increased 1 °C or more above the small increases that occurred during sham exposure. There was no evidence of either thermal or behavioral adaptation, nor were there indications of lasting microwave effects. Specific absorption rate (SAR) values, obtained from saline and tissue-simulating models, coupled with the performance decay finding at 38 and 46 mW/cm^2 indicate that special attention should be given to exposures of the head and extremities when establishing safety standards for human exposure.

ACKNOWLEDGMENTS

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*The animals used in this study were handled in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", Institute of Laboratory Animal Resources, National Research Council, DHEW, NIH Publication No. 80-23, 1980.

INTRODUCTION

It is of operational interest to know the microwave power level at which deterioration of human learning and performance begins, but experiments using man are not feasible. Consequently, non-human primate studies using operant conditioning techniques should yield information that could be extrapolated to man. Nelson (10) discovered that performance on a repeated-acquisition-of-chained-behavior task was impaired in restrained squirrel monkeys after being exposed for 30 minutes to 5.62 GHz pulsed microwaves at 53 mW/cm^2 intensity but performance was not impaired after exposure at 11 or 43 mW/cm^2. A mean increase in rectal temperature of 1.9 °C above control levels accompanied the behavioral effects, and no such effects were observed without concomitant hyperthermia. Galloway (6) reported effects using rhesus monkeys whose heads were irradiated (2.45 GHz, continuous wave) before the behavioral test sessions. Irradiation occurred for 2 minutes or until convulsions began. He found no effect on performance of a discrimination task, but under a repeated acquisition task irradiation at the highest power density consistently produced deficits in performance (reinforced on a variable ratio schedule). The effect on fixed ratio performance of the task was less marked. Schrot et al (14), using rats, found disruption of a repeated acquisition task after the animals had received 30 minutes of pulsed 2.8 GHz microwaves at both 5 and 10 mW/cm^2. de Lorge found behavioral and thermal effects in squirrel monkeys (2) at 50 mW/cm^2 and higher, and in rhesus monkeys (1) at 72 mW/cm^2. Both species were trained on a vigilance task and exposed to modulated 2.45 GHz microwaves.

The microwave studies by Nelson and Galloway involved monkeys irradiated prior to their behavioral measurements. The investigation reported here focuses upon the simultaneous interaction of microwave irradiation and learning and the power threshold at which any microwave effect occurred. All but two of Nelson's subjects were again used as experimental animals in the present experiment which extends his study in that behavioral measures were made while the monkeys were being irradiated.

METHOD

SUBJECTS

Four experimentally experienced male squirrel monkeys (*Saimiri sciureus*) about eight years old were maintained during the experiment at approximately 80% of their base line weights. The base line weights were means of a series of daily weighings while the monkeys were generously fed* twice each day, and were 712, 864, 852, and 1042 grams, respectively, for the four monkeys. They were individually caged with continuously available water.

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*Each feeding (one at 0700 hours and the other at 1500 hours) consisted of 15 Wayne Monkey Diet biscuits (produced by Allied Mills, Inc. Chicago, IL).

The vivarium cage room was kept at 24 ± 1 °C and had a light-dark cycle of 13-11 (lights were on at 0600-1900 hours). The monkeys had been previously used as subjects in a similar investigation of microwave effects (10).

APPARATUS

The outside dimensions of the anechoic exposure chamber, illustrated in Figure 1, measured 2.54 meters long, 1.94 meters wide, and 2.43 meters high. It was shielded with copper and then completely lined with 20.4 cm pyramidal absorber (AAP-8) obtained from Advanced Absorber Products, Amesbury, MA. A Styrofoam wall was placed between the absorber and the radiation source to permit better control of ambient temperature affected by convection heat produced in the back wall by absorption of microwave energy. The exposure chamber was equipped with a closed-circuit television camera.

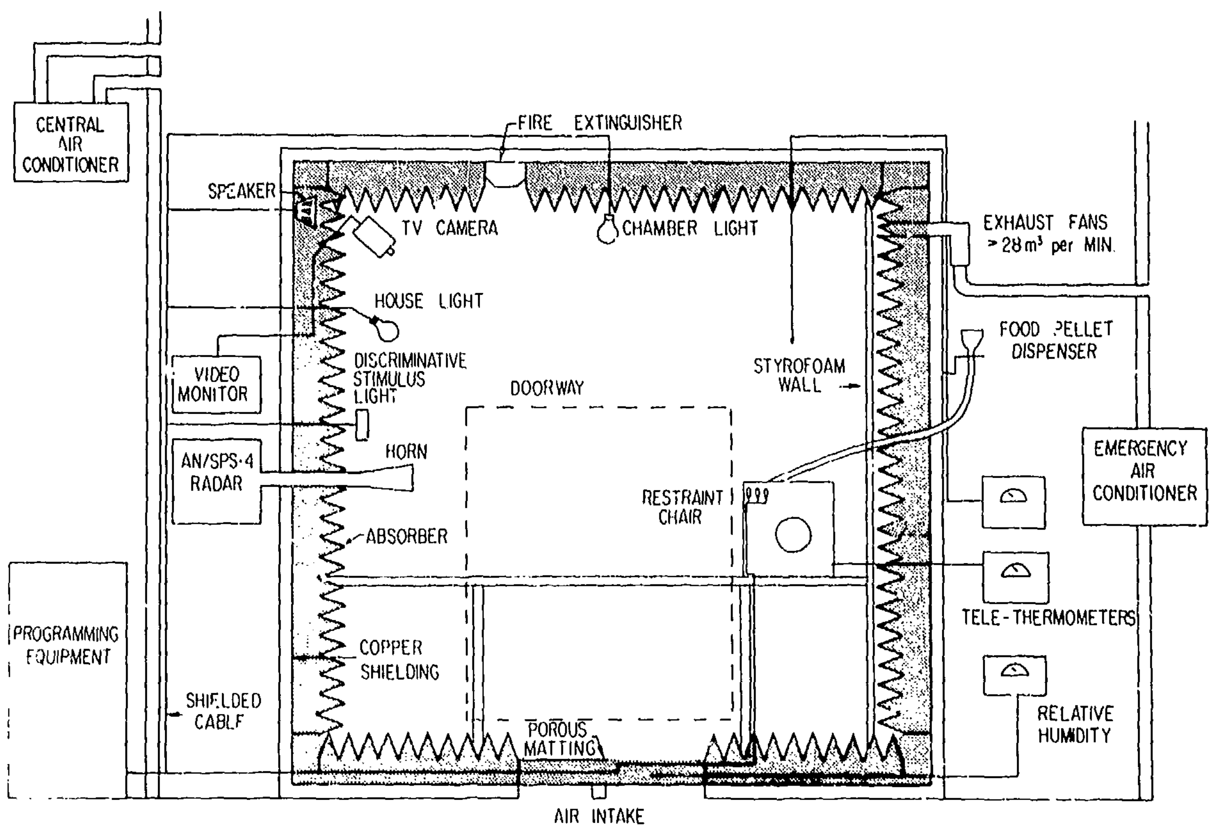

Figure 1

Diagram of the exposure chamber and ventilation system.

A radar set, AN/SPS-4 (Raytheon Manufacturing Company, Waltham, MA), was the source of 5.62 GHz microwaves pulsed at a repetition rate of 662 pulses per second with a pulse width of 2.0 μs. A wave guide connected a standard gain horn antenna, Narda model 642 (Narda Microwave Corporation, Plainsview, NY), to the source. The animal was restrained in an upright seated position in a Styrofoam chair (13) and was facing the microwave horn, which was vertically polarized. The animal was in the far-field, and his head was either 134.5 or 154.4 cm from the radiation horn, depending upon the desired power density.

The restraint chair shown in Figure 2 has three response levers placed to the subject's right, to his left, and approximately in front. These levers are respectively designated right (R), left (L), and center (C). Four lighted panels (4.3 x 5 cm in a plastic box 16 x 16 x 8 cm deep) were directly above and 10.5 cm behind the radiation horn opening. Associated with each visual stimulus was a discrete audible tone delivered from a speaker. A feeding tube terminal was connected to the left front side of the restraint chair. The food reinforcer was a 190 mg Noyes Precision food pellet (The P. J. Noyes Company, Lancaster, NH) delivered by the feeding tube into a trough in front of the monkey. General illumination in the chamber was provided by a white 150 watt light above the radiation horn when a session was not in progress, but during a session a white 25 watt overhead light was used. Luminance inside the chamber was 1.0 foot-candle with the 25 watt light and the stimulus light on. Masking noise was provided by a white noise generator and two ventilation fans. The combined sounds of the radar set, an audible discriminative stimulus, ventilation fans, and white noise produced a sound pressure level of 80 dB with the low tone which was at 1000 Hz, 74 dB with the middle tone at 1800 Hz, and 77 dB with the high tone at 2000 Hz.

Animal colonic temperature was measured with a Yellow Springs Instrument tele-thermometer, model 43TD (Yellow Springs Instrument Company, Yellow Springs, OH) and a thermistor probe (Yellow Springs Instrument temperature probe #402) inserted approximately 7 cm past the anal sphincter. The thermistor lead was taped to the base of the tail. In an adjacent room solid-state and electromechanical switching circuitry recorded responses and scheduled stimuli and response contingencies.

Air in the exposure chamber was circulated at a rate exceeding 28 m^3 per minute. A Yellow Springs Instrument temperature probe #405, located 40 cm above the subject's head, measured ambient temperature that varied between 23° and 25 °C, usually increasing during the day. Humidity was measured at the start and end of each experimental day. The overall average humidity was 53% with a slight decrease in humidity during the passing of the day (approximately 3%).

Using a Narda microline isotropic probe, model 8323, power density measurements were made for the three cardinal axes 15 cm in all 6 directions from the axes' intersection located at the approximate center of the monkey's face. With the Narda probe attached to the restraint chair, measurements were made at 2 cm intervals for the first 6 cm from the intersection and then, using a Styrofoam support device, the remaining measurements were taken at 10 and 15 cm from the axes' intersection. The power densities within a 4 cm cubic area corresponding to that area normally occupied by the monkey's head were averaged and this average is referred to as the power density illuminating the animals.

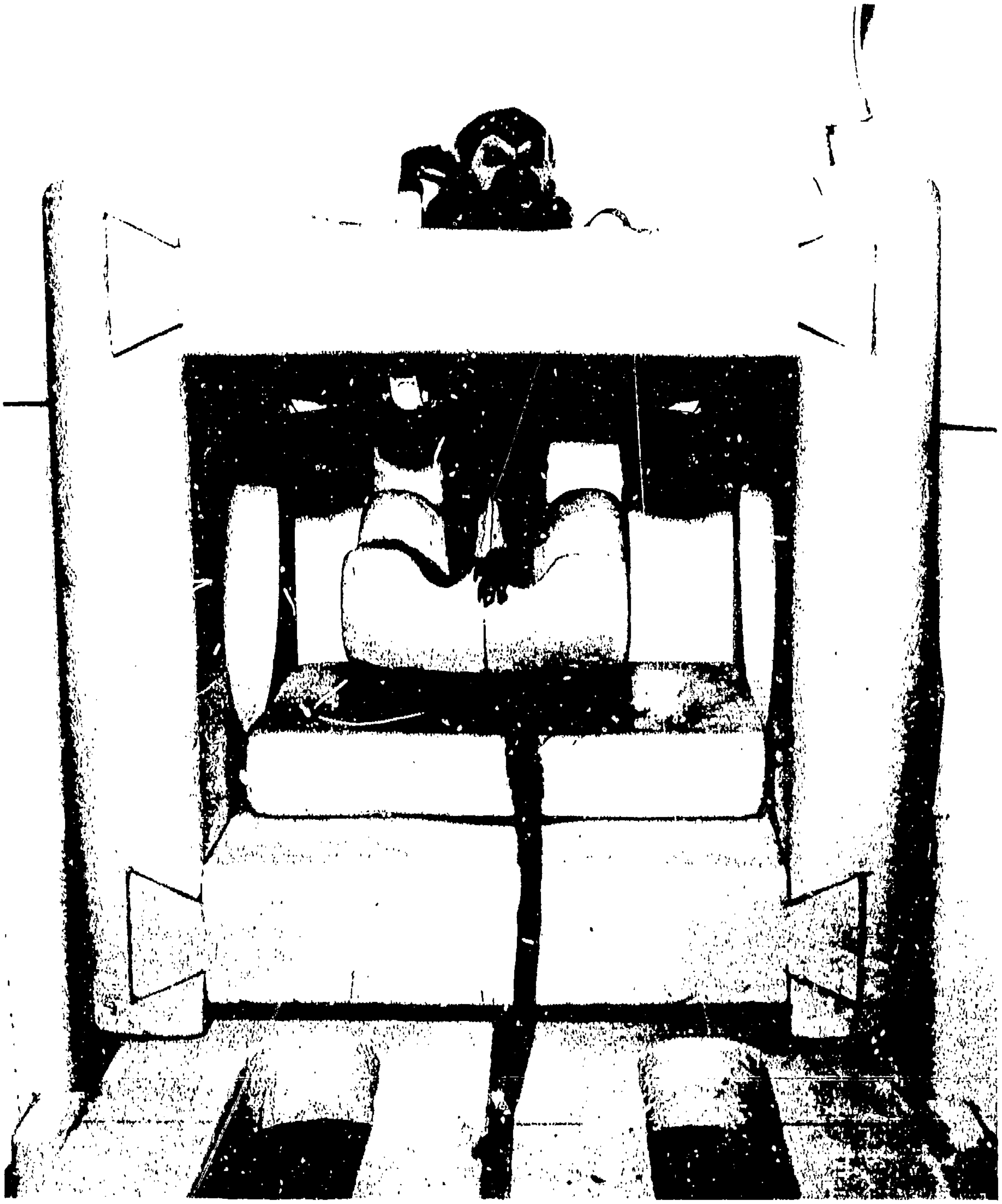

Figure 2. A squirrel monkey sitting in a restraint chair with three Teflon levers (right, left, and right of center). Insertion of the temperature probe was via a defecation aperture in the seat. Two terminal positions are possible for the food delivery tube, one beneath the plastic strap in front of the animal and the other position as shown to the monkey's left. In both cases the food pellet rolls into a trough in front of the monkey's head.

The initial position of the feeding tube terminal on the restraint chair was in front of the subject's head but it did not interfere with the animal's view of the stimuli. After completing the base line series and irradiations at 38 ± 14.6 mW/cm^2 and 17 ± 5.4 mW/cm^2 (in that order), it was found that moving the feeding tube terminal to the left of the subject's head, as shown in Figure 2, caused the field distribution near the head to change and the subsequent two power densities, 46 ± 7.3 mW/cm^2 and 32 ± 4.8 mW/cm^2 (in that order) were used to illuminate the animals at the same two chair positions as for the first two power densities.

PROCEDURE

The ultimate goal of training was to have a restrained monkey sequentially operate 3 levers with either hand in response to successive presentations of three different pairs of auditory-visual stimuli to obtain a food pellet. The association of a particular lever with an auditory-visual stimuli pair (discriminative stimulus, S^D) was changed each experimental session. Consequently, during the initial part of each session a new sequence or chain of stimuli-responses had to be learned. The series of experimental sessions demonstrated repeated acquisition of chained behavior. Training the four squirrel monkeys to do the task required 224 daily sessions excluding weekends and holidays.

Training began with food deprived monkeys in a restraint chair with a single response lever located in a standard sound-insulated animal conditioning chamber with white noise present and visual discriminative stimuli 78 cm in front of the subject's head. Eventually the conditioning process proceeded to two and then three response levers in the microwave chamber, with the subject's head 134.5 cm from the center of the antenna aperture. Food pellets were delivered (reinforcement, RF) following a series of correct stimulus-lever actions. The original criterion for advancing from one stage of training to the next was the animal's achievement of 70% or greater correct responses during an operant session. This performance criterion was compromised in the later stages especially in the case of subject 36. The final stage of training produced a subject who had a 90-min experimental session that was subdivided in the following manner: a 15-min pre-session with inoperative levers; a 60-min behavioral portion wherein the monkey performed the repeated acquisition task; a 15-min post-session with inoperative levers. The repeated acquisition of chained behavior involving 3 S^D's and a response (R^D) on each of 3 different levers may be visualized in the following way:

$$S^D1 \longrightarrow R^D1 \longrightarrow S^D2 \longrightarrow R^D2 \longrightarrow S^D3 \longrightarrow R^D3 \longrightarrow RF$$

The S^D sequence (yellow light and low tone, green light and middle tone, red light and high tone) was always the same, but the R^D sequence (left, center, right) was changed each session. Only a correct sequence of responses resulted in delivery of a food pellet. Neither the first nor the last R^D of a sequence was ever repeated in the same place in a succeeding session. Subjects 40 and 52 had identical reinforcement schedules. For these subjects an R^D consisted of a fixed ratio of 2 responses, FR 2, on each lever. Subject 36's schedule was similar to theirs except he was on a FR 1 to change the S^D, i.e., an R^D consisted of one lever response. Subject 37's schedule was the same as 40 and 52 but the chamber light was on for the entire session because of his failure to respond in the absence of the chamber light. A 7-s time-out (TO) was imposed when an error was made, that is, the S^D and chamber lights were off for seven seconds. Lever responses had no effect during the time-out. Incorrect responses did not reset the sequence. There was also an 0.8-s period after each correct FR during which lever responses had no consequences.

After all subjects had achieved 12 successive sessions of consistent behavior, the end of training session 224, 38 experimental sessions were conducted. A sham session preceded and followed each microwave exposure session. The initial six sessions were considered base line data with which successive sham sessions were compared. An initial incident power density of 38 mW/cm^2 was selected based on other workers' experiences (1, 2, 10) and 4 irradiation sessions were conducted.* Successive groups of sham and irradiation exposures were conducted at 17, 46 and 32 mW/cm^2 power levels, in that order. Irradiation was always applied during the middle 60-min behavioral portion of a session. For sham sessions the only difference was the absence of irradiation; the radar set was on but the magnetron was not activated.

Experiments were conducted five days a week, and each day the squirrel monkeys were placed in the microwave exposure chamber in the same order. Colonic and ambient temperatures were monitored throughout the entire experiment. About 15 minutes were required to remove one monkey from the chamber, return it to the vivarium and prepare another monkey. Total time devoted to a daily session with one animal was about two hours.

Throughout training, attention was given to the single session development of learning and subsequent performance of correct lever responding; hence, learning curves (graphic representations of learning as a function of time) were plotted daily. Three of the monkeys (37, 40 and 52) did attain classic learning curves, but 36 never did. Instead, 36's percent correct response curve was a straight line, usually with a declining slope and indicated chance performance, with less than 50% correct responses.

To gain some idea as to what thermal effects the microwaves would evoke in a 750 g animal, models of balloon-held water (Figure 3) and rubber-glove-held muscle-simulating material were exposed to 46 mW/cm^2 incident radiation with the model's head in the far-field 134.5 cm from the radiation horn and in the animal position in the restraint chair. The dosimetric techniques of Olsen (11) and Guy (7) were followed. Temperature measurements in the irradiated models were performed with a Vitek model 101 Electrothermia Monitor (Vitek, Inc, Boulder, CO).** The water model

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* Subject 36 was given only 3 sham and 3 irradiation exposures at 38 mW/cm^2.
** Calibration checks were made at six month intervals by comparing the Vitek temperature probe with a Hewlett-Packard 2802A platinum probe thermometer (Hewlett Packard, incorporated, Palo Alto, CA) in a stirred, insulated water bath.

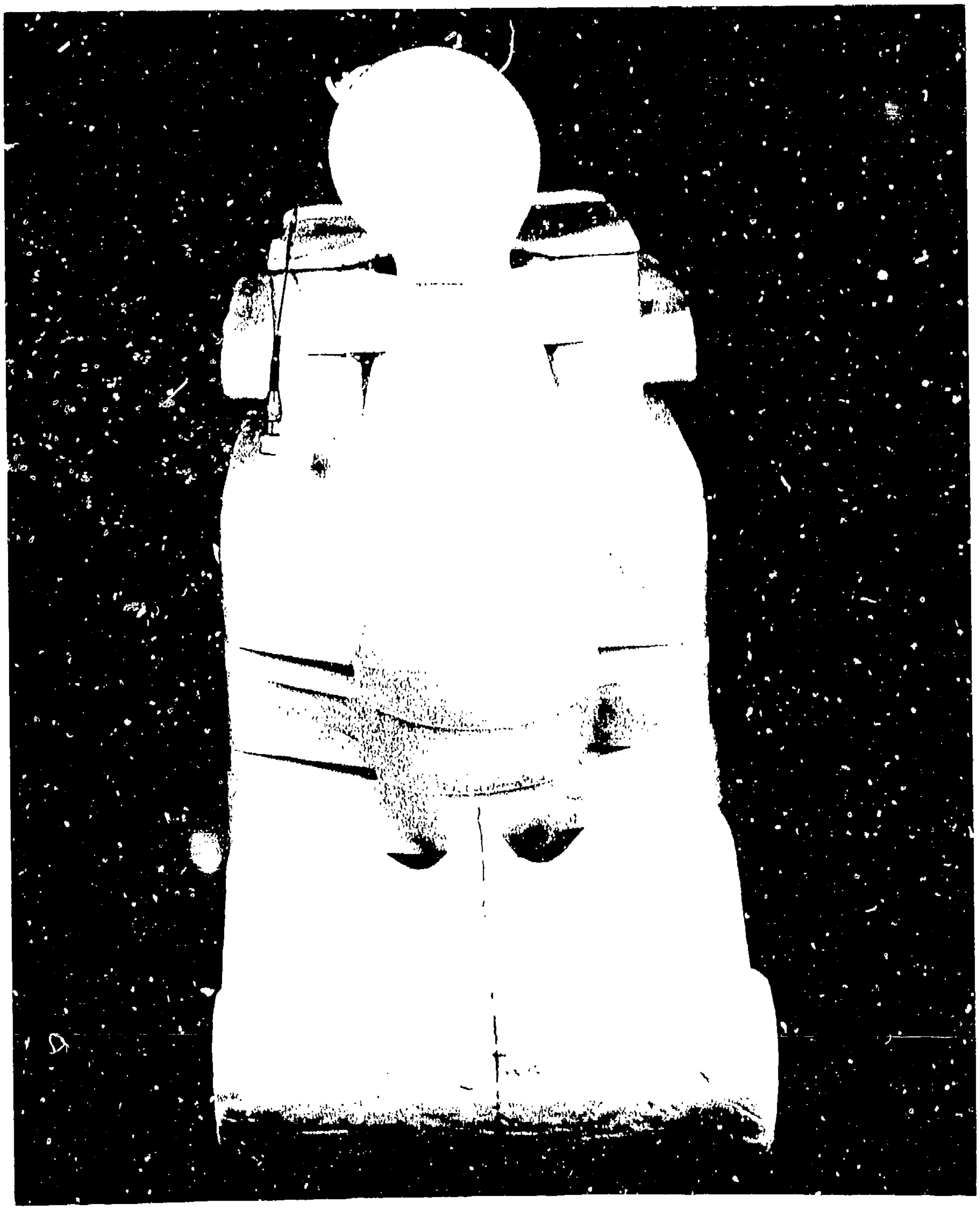

Figure 3. Ventral view of a squirrel monkey 750 g ballon-held water model (head and torso only) occupying the subject's position in the removable portion of the restraint chair. Temperature sensor lead projects from closed balloon opening and detects temperature of water approximately in the middle of the torso area.

temperature measurements were made with the Vitek probe located at the approximate center of the model abdomen. For the model of muscle-simulating material the temperature probe was inserted through holes in the rear of the model at selected locations. The voltage-analogue output of the temperature probe was recorded. The graphical records of the change in temperature (ΔT) versus time were used to calculate the averaged specific absorption rate, SAR, by standard calorimetric methods (4, 5, 8, 9) where care was taken to use only the initial linear slope of the ΔT graph. The normalized SAR was 0.18 (W/kg)/(mW/cm^2) for the water model and 0.20 (W/kg)/(mW/cm^2) for the muscle-simulating model as calculated by the formula (8) $P_L = 4186\ \rho c\ \Delta T/\Delta t$, where c = tissue specific heat in cal/(g · °C), 1.0 for water and 0.86 for muscle simulating material; ρ is density in g/cm^3, 1 in both cases; ΔT = temperature rise in °C; Δt = duration of irradiation in seconds; and P_L = W/kg).

RESULTS AND DISCUSSION

Group mean percent correct responses per reinforcement as a function of time and power density along with mean colonic and ambient temperatures are shown in Figure 4 for the four monkeys. Figure 4 response curves show that there was no large behavioral effect in the initial portion of a session when the new lever response sequence was being learned, but beginning at 38 mW/cm^2 performance began to decrease during the latter portion of the session. The learning period was usually during the first 20-25 minutes of the behavioral portion of each session as indicated by where the maximum percent correct responses per reinforcement occurred; thereafter performance deteriorated regardless of exposure conditions. These mean curves show that only in the case of the highest irradiation condition was rate of learning decreased.

The colonic temperature graphs of Figure 4 indicate the progressive increase of temperature during an irradiation with a post-session decrease in temperature. It was noted that once the colonic temperature had shown an increase of 1-2 °C during the irradiation session, the number of correct responses began to decrease more than during sham sessions, and this decrease usually occurred 30-45 minutes after the start of the 38 and 46 mW/cm^2 sessions. Note in Figure 4 that during base line (0 mW/cm^2) and sham sessions the colonic temperature increased about 0.5 °C and that some increase occurred during the behavioral portion alone.

Ambient temperatures shown in Figure 4 began at about 24.5 °C and gradually decreased to around 23 °C when the behavioral portion began. The temperature remained at that level until the later portion of a typical irradiation session when it gradually increased about 0.5 °C, and then decreased back to 23 °C at 15 minutes post irradiation. Sham session ambient temperatures remained at about 23 °C from the start of the operant portion until the microwave chamber door was opened for removal of the animal.

Figure 5 gives the mean colonic temperature increase for the four monkeys during the behavioral portion of each consecutive session. These data demonstrate the stable temperature rise in sham sessions, the non-linear relationship between temperature rise and intensity of radiation,

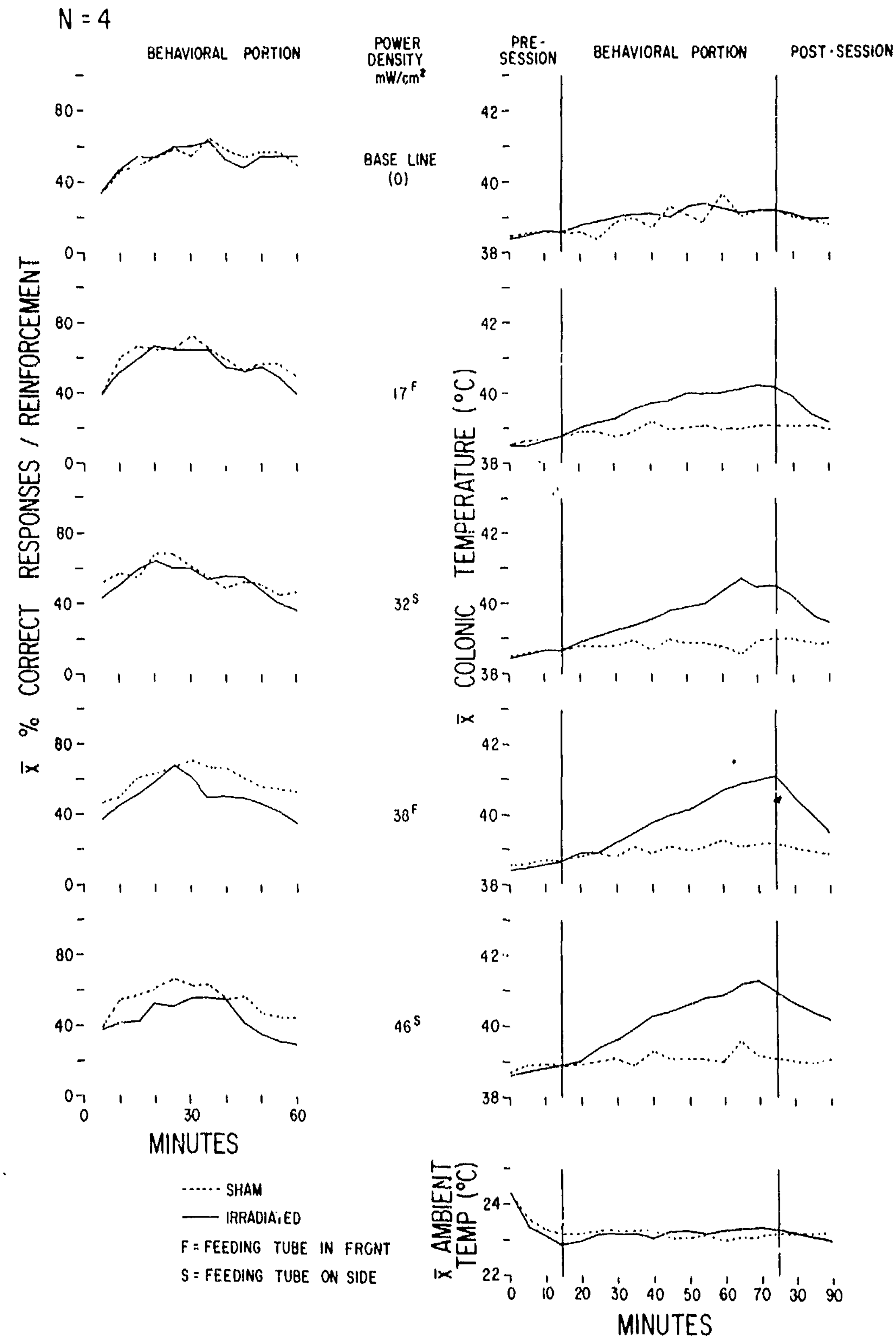

Figure 4

Composite graphic presentation showing the effect of increasing microwave power density on conditioned behavior-responding and on colonic temperatures for a group of four squirrel monkeys. Each graphic point represents five minutes of averaged data.

and also the greater temperature increase during the initial session of exposure at all levels except 38 mW/cm^2.

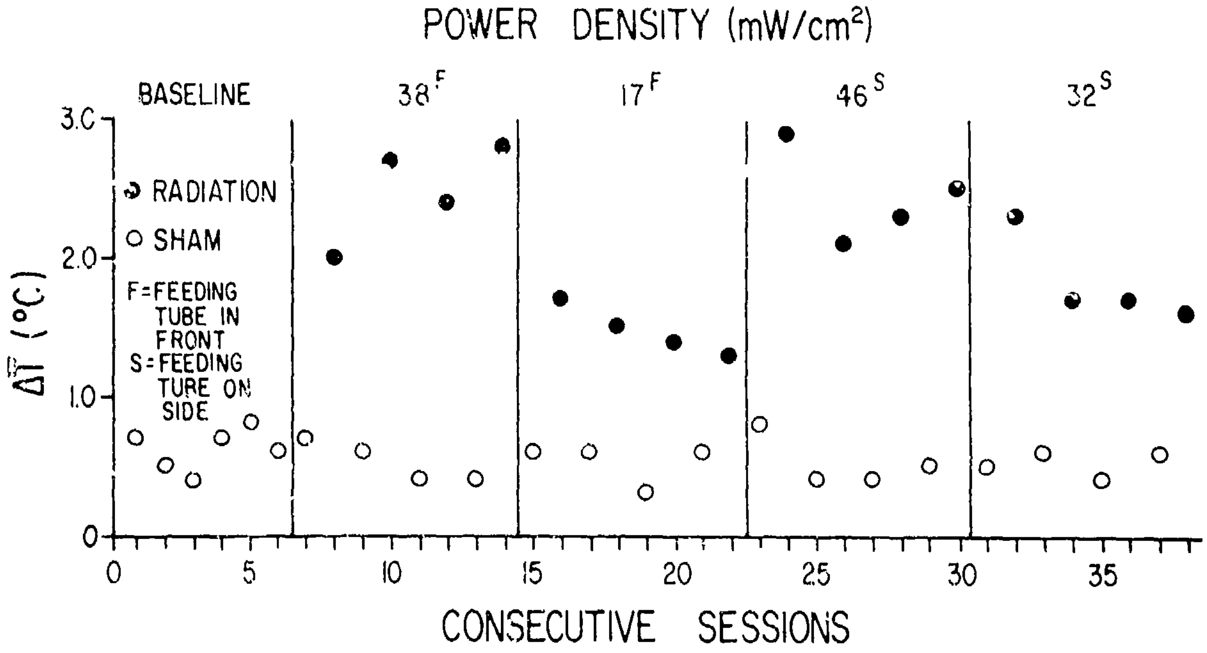

Figure 5

Average increase of colonic temperature for four squirrel monkeys during each behavioral portion of the series of microwave experiments.

The characteristic cumulative response records of subject 52, shown in Figure 6, most nearly approximate the group averages graphically displayed in Figure 4. Consequently he was considered a representative behavioral and thermal responder. Each cumulative record is of the 60-min behavioral portion of a session. The response pen reset at 234 responses or at the end of a session. The pen was deflected downward with each wrong response and stepped upward with each correct response. The hash marks on the horizontal line below the response record indicate when a food pellet was delivered. Essentially at 46 mW/cm^2 subject 52 had a large performance decrement (less responding with long pauses) at the end of that session but none during any sham session and only a slight, almost imperceptible, decrease during irradiation at power densities of 17 and 32 mW/cm^2. However, at 38 mW/cm^2 this animal responded throughout the session at lower rates and made many more errors than during its sham session comparison.

Figure 7 has subject 36's mean behavioral response and colonic and ambient temperature changes. These data show that he did not learn; he obtained food pellets essentially "by chance" (less than 50% correct responses) and during both sham and irradiation sessions the percent correct responses usually decreased. At the 17, 38, and 46 mW/cm^2 power densities, there were sessions during which subject 36 remained inactive for the first portion (1-18 minutes) of the behavioral session, and once he began responding, the remaining part of the session was marked by relatively

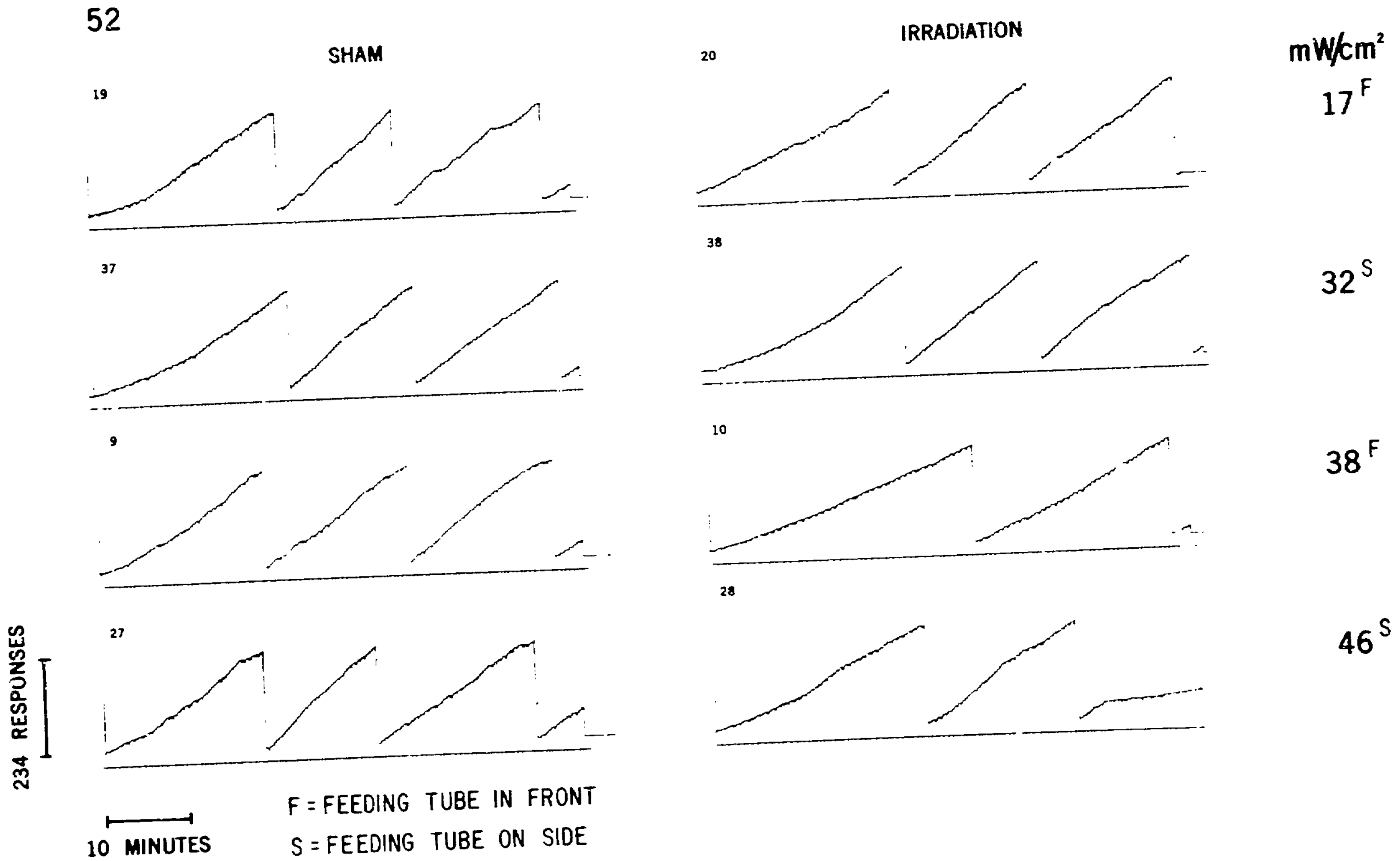

Figure 6. Representative cumulative records of squirrel monkey 52 for the series of pulsed microwave experiments at 5.62 GHz. Each is a 60 minute session of the repeated acquisition operant paradigm. The response pen was reset at 234 responses or at the end of a session. The pen was deflected downwards with each wrong response and stepped upwards with each correct response. A downward movement of the pen on the horizontal line at the bottom indicated delivery of a food pellet (reinforcer). Experiment session number is at upper left corner of each record.

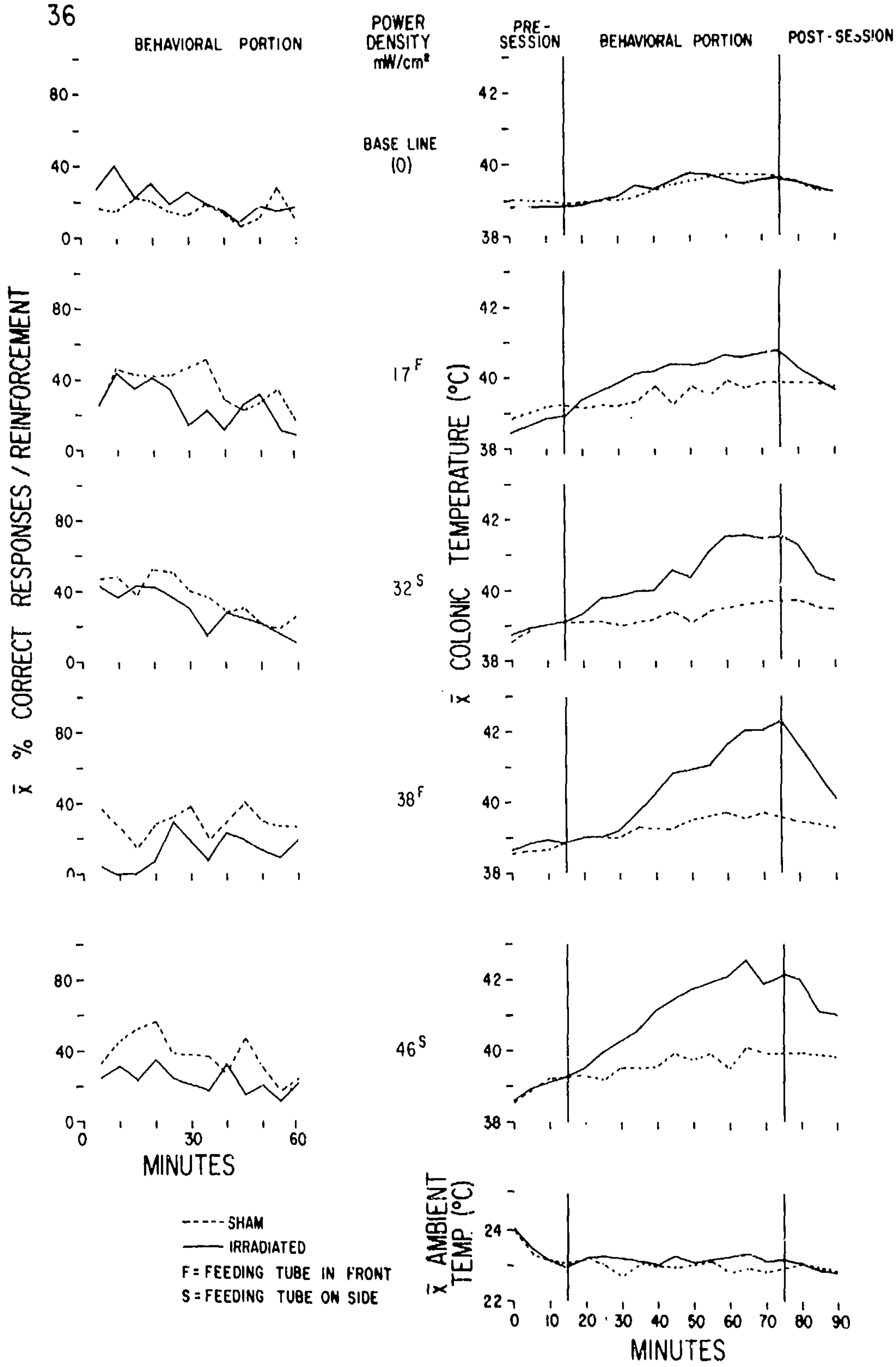

Figure 7. Composite graphic presentation showing the effect of increasing microwave power density on responding and on colonic temperatures for squirrel monkey 36. Each graphic point represents five minutes of averaged data.

fewer correct responses than during sham sessions. The colonic temperature graphs in Figure 7 reveal an increasing difference between starting and ending values as power density was increased, but there seems to be no striking relationship between increasing colonic temperature and operant performance. Subject 36's records demonstrate slight performance deficits along with thermal effects at 38 and 46 mW/cm^2. In fact, subject 36 seems to be sensitive, by thermal criteria, to all intensities of irradiation to which he was exposed. During one 46 mW/cm^2 session subject 36's colonic temperature went to 43.6 °C, and the session was aborted after 54 minutes.

Intertest interval did not seem to increase the total number of responses and number of food pellets delivered to the monkeys. In contrast, Premack and Bahwell (12) described an increased number of responses when the intertest interval was widened for a cinnamon cebus monkey. The data for each of our animals show that in most cases when several days, even as many as 9 and 11 days separated sessions, there was no significant change in number of responses.

Our data reveal that total responding and food pellet deliveries for all subjects seemed to be maintained at a constant range with consistent individual differences during the microwave irradiation series. Subject 36 had less responding at 38 and 46 mW/cm^2, but there were no differences between sham and irradiation sessions at 17 and 32 mW/cm^2. Subject 37 demonstrated the same relative number of total responses for all sham and irradiation sessions. Considering all of subject 37's data, only an inspection of his cumulative records reveals a performance effect, which was during 46 mW/cm^2 irradiation. Subject 40's responding was reduced at each intensity of irradiation, and subject 52's was only reduced at 46 mW/cm^2. Therefore, even with a wide variation in microwave effect on total responses, it is evident, in general, that the group of four subjects was undeniably affected at 46 mW/cm^2 as shown by a reduction in responding. Consequently, the threshold of this performance effect for the group should be somewhat lower than 46 mW/cm^2.

At 46 mW/cm^2, averaged SAR values calculated from the 16 locations in the muscle-simulating model were variable with the highest values in the legs, arms, and neck, and the lowest values in the abdominal region. Realizing the approximate nature of these calculations the values were combined to provide a single overall average of 9.2 W/kg. This value is close to that obtained at one location in the water model, 8.4 W/kg. The averaged SAR compared to the resting metabolic rate of 4.47 W/kg (4) revealed that the water model absorbed radiation at about twice the rate of metabolic heat production in a resting squirrel monkey. The normalized SAR of the water model, 0.18 (W/kg)/(mW/cm^2), is similar to that obtained on rats irradiated at 5.62 GHz (3).

CONCLUSIONS

This report confirms the performance decay findings of Nelson (10), Galloway (6), and de Lorge (1, 2), who also irradiated operant conditioned monkeys. Our data support previous conclusions (2, 10) that in squirrel monkeys the threshold at which errors in performance increase is between 38 and 46 mW/cm^2 and that the simultaneous increase in colonic temperature is

1° - 2 °C greater than corresponding increases during sham exposures. Successive scheduled sessions, with each irradiation session separated by at least one sham session, gave no indication of temperature or performance adaptation. One subject showed several instances of not responding during the early portion of an irradiation session but this did not continue for all of the series. No cumulative microwave effects were seen.

From SAR values derived from calorimetry of irradiated water and muscle-simulating models, it is believed that even though average whole body SAR values are only about twice the resting metabolic rate there are local heat concentrations with greater SAR values, especially in the head and other extremities. This means that safety considerations should not be limited to protection based on the overall average energy deposition but must also take into account the head and limbs which may be affected by microwaves to a greater extent.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Navy personnel assigned to duties in the vicinity of microwave producing devices are subject to possible hazards if the irradiation is of sufficient strength. Of critical need are data to establish safety standards for human exposure to microwaves. Such information cannot be gained from human studies because of potential hazards, but fairly reliable and valid results from nonhuman primate experiments may be extrapolated to man and then used for setting human safety standards. To this end squirrel monkeys were

20. ABSTRACT (continued)

trained on a task that required repeated learning and then they were irradiated with microwaves while performing the task.

Four male squirrel monkeys trained to perform a repeated acquisition of chained behavior demonstrated performance decay, while being exposed to pulsed 5.62 GHz microwave radiation in the far-field situation at power densities of 38 and 46 mW/cm^2. There was little, if any, effect on learning or performance at 17 and 32 mW/cm^2. The performance effect became evident when the monkey colonic temperature increased 1 °C or more above sham levels. There was no evidence of either thermal or behavioral adaptation nor were there indications of permanent microwave effects. Specific absorption rate (SAR) values obtained from saline and flesh-simulating models coupled with the performance decay finding at 38 and 46 mW/cm^2 indicate that special attention should be given to head and extremities when making human safety standards.